Citation:

Saposnik G, Ray JG, Sheridan P, McQueen M, Lonn E; Heart Outcomes Prevention Evaluation 2 Investigators. Homocysteine-lowering therapy and stroke risk, severity, and disability: Additional findings from the HOPE 2 trial. *Stroke*. 2009; 40 (4): 1,365-1,372.

PubMed ID: 19228852

Study Design:

Randomized clinical trial

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

POSITIVE: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To determine whether homocysteine-lowering therapy reduces the risk of ischemic and hemorrhagic stroke, as well as stroke-related disability.

Inclusion Criteria:

- Men and women 55 years of age or older, irrespective of their homocysteine levels from countries:
  - With mandatory folate fortification of food (Canada and the United States)
  - Without mandatory folate fortification (Brazil, western Europe and Slovakia)
- History of:
  - Stroke
  - Transient ischemic attack (TIA) with objective evidence of ischemic ischemic cerebrovascular disease or with endarterectomy
  - Vascular disease (coronary, cerebrovascular or peripheral vascular)
  - Diabetes and additional risk factors for atherosclerosis.

Exclusion Criteria:

Patients who were taking vitamin supplements containing more than 0.2mg of folic acid per day.

Description of Study Protocol:

Recruitment

Subjects were recruited from 145 participating centers in 13 countries:

- Canada (N=3,568)
- United States (N=414)
- Brazil (N=265)
- Western Europe (N=426)
- Slovakia (N=849).

Design

Randomized clinical trial.

Blinding Used

Unclear as to whether the study with single or double blind. The discussion indicates that the study was "masked."

Intervention

- Participants were randomized (central randomization) to receive a once-daily supplement containing 2.5mg of folic acid, 50mg of vitamin B_6, and 1.0mg of vitamin B_{12}, or matching placebo, for five years
- No request was made to unmask treatment allocation for any given participant
- Participants were assessed in study clinics at six-month intervals.

Statistical Analysis

- The distribution of homocysteine was statistically significantly skewed so these data were log-transformed and inverse transformations were used to generate geometric mean values
- All analyses were by intention-to-treat, comparing the effect of homocysteine-lowering therapy against placebo. Binary stroke outcomes were analyzed by Cox proportional hazards regression models and risk estimates were expressed as a crude HR and 95% CI. Survival curves were estimated according to the Kaplan-Meier procedure and compared between treatment groups using a log-rank test. In the rare (less than 1%) circumstance that an individual could not be assessed at a clinic visit or contacted by telephone, the individual was considered to be free of a cerebrovascular event at that point in time. If lost to follow-up, then a participant was censored at the time of last contact. Censoring also occurred when a participant died or experienced any form of stroke, but not a TIA. Risk of stroke in pre-specified subgroups, including sex, those older and younger than the median age of the study participants, those with and without a history of hypertension, diabetes, smoking, coronary artery disease, TIA/stroke, cholesterol levels above or below the median of study

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participants, use of lipid-lowering therapy, use of antithrombotic agents, region (mandatory food fortification with folic acid vs. non-fortified areas), and quartiles of homocysteine levels was also assessed. In addition, a post-hoc analysis using a multivariable Cox proportional hazards models to evaluate the effect of homocysteine-lowering vitamin therapy on stroke adjusted for age, sex, and extended use of an antihypertensive, antiplatelet and lipid-lowering agents (model 1) and age, sex and geographic region (model 2) was conducted
- Among persons with an incident stroke, the net mean difference in change in neurological deficit from initial presentation to that at 24 hours was compared between homocysteine-lowering therapy and placebo groups using a Wilcoxon rank sum test. The trend in the modified Rankin scores at hospital discharge (or seven days after stroke onset) was assessed using a Mantel Haenzel $\chi 2$ test. Modified Rankin scores were categorized into two levels of function: Independence (a score between zero and two) and dependence (a score of three to six). The risk of being functionally dependent after stroke was expressed as an OR and 95% CI, comparing persons who received homocysteine-lowering therapy to those assigned placebo.
- Statistical significance was set at a two-sided $P<0.05$ for all analyses, which were performed using SAS version 9.1 (SAS Institute Inc.).

Data Collection Summary:

Timing of Measurements

- Measurements of study endpoints, side effects and adherence were repeated at six-month intervals
- Adherence to treatment was assessed by interview and pill count.

Dependent Variables

- Plasma homocysteine after overnight fast, by fluorescence polarization immunoassay (Abbott IMX; Abbott Laboratories, Inc.)
- All stroke events, but not TIA, were centrally adjudicated by the Adjudication Committee using pre-specified definitions of outcomes and all available supporting source documentation. TIA was excluded from all stroke outcomes. A disabling stroke was based on a modified Rankin score of three to five, equivalent to having moderate to severe disability. A stroke was defined as a focal neurological deficit lasting more than 24 hours. Computed tomography or magnetic resonance cranial imaging was obtained for 92% of participants with an ischemic stroke. Among persons with an incident stroke, the following symptoms were recorded at stroke onset and at 24 hours thereafter:
  - Change in the level of consciousness
  - Ocular or visual symptoms (diplopia or amaurosis fugax)
  - Motor weakness
  - Sensory symptoms
  - Dysarthria or dysphasia
  - Dysphagia
- A higher number of symptoms suggested a worse neurological deficit
- Separately, stroke-related disability at discharge from hospital or at seven days after stroke (whichever came first) was determined using the modified Rankin scale, graded from zero to six, as described in Supplementa.

Independent Variables

Number of cases of stroke.

Control Variables

- Baseline demographic data, medical history and medication use, including current use of anticoagulant therapy, were recorded for all participants at study entry. History of stroke or TIA was also documented
- After an overnight fast, blood was obtained for measurement of baseline plasma homocysteine from 3,306 randomly selected participants.

Description of Actual Data Sample:

- *Initial N:* 5,522 (28% women)
- *Attrition:* 21 participants in the active arm and 16 in the placebo arm were lost to follow-up or withdrew from the study, but all were enrolled for at least two years and were included in the final analysis, censored for duration of observation
- *Mean age:* 69 years
- *Ethnicity:* Participants were from Canada, US, Brazil, Western Europe and Slovakia
- *Other relevant demographics:*
  - There was no significant differences in baseline characteristics between those receiving placebo and therapy
  - The geometric mean homocysteine concentration at baseline, measured among 3,306 participants, was 11.5µmol per L in both groups. In the active group, the mean concentration was 2.1µmol per L lower in countries with folic acid food fortification than in those without fortification; in the placebo group, this difference at baseline was 2.3µmol per L. At the end of the trial, the mean homocysteine concentration was 9.3µmol per L in the vitamin therapy group and 12.3µmol per L in the placebo group, indicating a decrease of 2.2µmol per L and an increase of 0.80µmol per L, respectively.
- *Anthropometrics:*

Table 1. Baseline Characteristics of Study Participants

Baseline Characteristics*	Homocysteine-Lowering Therapy (N=2,758)	Placebo (N=2,764)
General characteristics		
Mean (SD) age, year	68.8 (7.1)	68.9 (6.8)
Female	796 (28.9)	763 (27.6)
Ethnicity, non-white	106 (3.8)	109 (3.9)
Living in North America	1,988 (72.1)	1,994 (72.1)

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Cardiovascular risk factors		
Coronary artery disease	2,285 (82.8)	2,315 (83.8)
TIA	158 (5.7)	154 (5.6)
Stroke	241 (8.7)	251 (8.9)
TIA or stroke	341 (12.4)	343 (12.4)
Peripheral artery disease	216 (7.8)	169 (6.1)
Hypertension	1,542 (55.9)	1,497 (54.2)
Dyslipidemia	1,524 (55.3)	1,526 (55.2)
Diabetes mellitus	1,122 (40.7)	1,087 (39.3)
Current smoking	306 (11.1)	327 (11.8)
Baseline medication use		
Antiplatelet agent	2,148 (77.9)	2,224 (80.5)
Oral anticoagulant	227 (8.2)	193 (7.0)
Lipid-lowering agent	1,627 (59.0)	1,690 (61.1)
Estrogen replacement therapy among women	137 (17.2)	130 (17.0)
Multivitamin supplement	331 (12.0)	307 (11.1)
Baseline physical measures		
Mean (SD) heart rate, bpm	68.7 (11.2)	68.9 (11.5)
Mean (SD) systolic blood pressure, mmHg	138.8 (21.7)	138.9 (23.4)
Mean (SD) diastolic blood pressure, mmHg	77.4 (11.8)	77.5 (11.7)
Mean (SD) BMI, kg/m^2	29.6 (16.4)	29.7 (21.1)
Baseline biochemical measures		
Mean (SD) serum total cholesterol, mmol per L	4.83 (1.01)	4.78 (0.98)
Mean (SD) serum LDL cholesterol, mmol per L	2.73 (0.85)	2.68 (0.83)
Mean (SD) serum HDL cholesterol, mmol per L	1.21 (0.36)	1.18 (0.34)
Mean (SD) serum triglycerides, mmol per L	2.02 (1.35)	2.04 (1.29)
Mean (SD) serum glucose, mmol per L	7.15 (3.22)	6.97 (2.88)
Geometric mean (SD) plasma homocysteine concentration, µmol per L ¡	11.5 (0.80)	11.5 (0.80)

*Data are presented as a number (percentage) unless otherwise indicated.

¡A total of 3,306 participants underwent measurement of plasma homocysteine at baseline.

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein.

- *Location:* Individuals were recruited from 145 participating centers within 13 countries, including Canada (N=3,568), US (N=414), Brazil (N=265), Western Europe (N=426) and Slovakia (N=849).

Summary of Results:

- There were 258 (4.7%) strokes in total. 111 (4.0%) patients in the homocysteine-lowering group and 147 (5.3%) in the placebo group had a stroke (HR, 0.75; 95% CI, 0.59 to 0.97; absolute risk reduction, 1.3%; Table 2)
- The corresponding risk of ischemic stroke (HR, 0.81; 95% CI, 0.60 to 1.09) and hemorrhagic stroke (HR, 0.80; 95% CI, 0.32 to 2.02) tended to favor vitamin therapy, but not significantly so (Table 2)
- The relative risk of stroke was most reduced among those who baseline homocysteine concentration was in the highest quartile
- The effect of therapy was greater among subgroups of person younger than age 70 years, with untreated hyperlipidemia, those not receiving an antiplatelet agent and in persons with hyperhomocystinemia or residing in a country without folic acid food fortification.

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Table 2. Stroke Outcomes Comparing Homocysteine-Lowering Therapy vs. Placebo

	N (Incidence Rate per 100 Person-Years)		
Outcome*	Homocysteine-Lowering Therapy (N=2,758)	Placebo (N=2,764)	HR (95% CI) Homocysteine-Lowering Therapy vs Placebo
Any stroke	111 (0.88)	147 (1.15)	0.75 (0.59–0.97)
Ischemic stroke	79 (0.62)	98 (0.77)	0.81 (0.60–1.09)
Hemorrhagic stroke	8 (0.06)	10 (0.08)	0.80 (0.32–2.03)
Non-fatal stroke	84 (0.66)	117 (0.92)	0.72 (0.54–0.95)
Fatal stroke	27 (0.21)	30 (0.24)	0.91 (0.54–1.53)
Disabling stroke	26 (0.21)	41 (0.32)	0.64 (0.39–1.04)
Fatal or disabling stroke	48 (0.38)	63 (0.49)	0.77 (0.53–1.12)

*If a participant had more than one outcome during the study period, only the first event was included in this analysis.

Table 3. Change in Neurological Deficit at the Onset of Stroke Symptoms and at 24 Hours Thereafter, Comparing Homocysteine-Lowering Therapy vs Placebo

	Mean (SD) Neurological Deficit Score*						
	At Initial Onset of Stroke Symptoms		At 24 Hours After Onset of Stroke Symptoms		Change Between 24-Hour and Stroke Symptoms at Initial Onset		
Stroke Type	Homocysteine-Lowering Therapy	Placebo	Homocysteine-Lowering Therapy	Placebo	Homocysteine-Lowering Therapy	Placebo	P¡
Any stroke	2.2 (1.4)	2.3 (1.5)	1.4 (1.4)	1.5 (1.4)	–0.85 (1.4)	-0.77 (1.4)	0.45
Ischemic	2.4 (1.3)	2.5 (1.5)	1.4 (1.2)	1.6 (1.4)	–0.96 (1.4)	-0.93 (1.5)	0.69
Hemorrhagic	1.6 (1.9)	2.2 (1.6)	1.4 (1.9)	1.8 (1.3)	–0.25 (0.89)	-0.4 (1.3)	1.00

*Defined by the number of symptoms as follows:

- Change in level of consciousness
- Ocular or visual symptoms
- Motor weakness
- Sensory symptoms
- Dysarthria or dysphasia
- Dysphagia.

A higher score indicates a greater neurological deficit.

¡Wilcoxon rank sum test comparing the net mean difference in the change in the neurological deficit score between homocysteine-lowering therapy vs. placebo.

Author Conclusion:

- In conclusion, combined daily administration of 2.5mg of folic acid, 50mg of vitamin B_6, and 1.0mg of vitamin B_{12} for five years had a modest, but beneficial effect on stroke prevention or fatal stroke among a population at high risk for cardiovascular disease
- Ongoing clinical trials should help determine whether folic acid and B vitamin supplements are efficacious in reducing stroke risk and severity and which patient subsets are most likely to derive benefit from this treatment.

Reviewer Comments:

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The author's noted the following limitations:

- *Only approximately 12% of participants had a historyof stroke or TIA. Nevertheless, the trial a fairly large number of stroke events*
- *Also, because the mechanisms for ischemic stroke were not captured, they cannot comment whether homocysteine-lowering therapy may differentially impact on large vs. small artery disease*
- *The non-significant reduction in disability at seven days or at discharge is not surprising, because stroke recovery often requires weeks rather than days, and this would lower the ability to detect a clinically important difference, especially given the statistical power of only 30% for ischemic stroke and 42% for disabling stroke in the current trial*
- *The original HOPE-2 trial was designed and powered to detect a proportional reduction in the risk of cardiovascular death, myocardial infarction and stroke, rather than just stroke or one of its subtypes*
- *Approximately two-thirdsof the study patients were recruited from countries with folate food fortification, which may impact on the ability of the study intervention to further lower homocysteine levels. However, compared to the placebo group, homocysteine levels at study end were approximately 25% lower in the active treatment group*
- *The results of this secondary analysis of the HOPE-2 trial may only be applicable to adults older than 55 years with atherosclerosis.*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes
Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	No
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	Yes
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes

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	4.1.	Were follow-up methods described and the same for all groups?	Yes
	4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
	4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	No
	4.4.	Were reasons for withdrawals similar across groups?	???
	4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**		Yes
	5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	Yes
	5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
	5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
	5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	Yes
	5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**		Yes
	6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
	6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
	6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	???
	6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
	6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	Yes
	6.6.	Were extra or unplanned treatments described?	Yes
	6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
	6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**		Yes
	7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
	7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
	7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	???
	7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
	7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
	7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
	7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**		Yes
	8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
	8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
	8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
	8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A

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	8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
	8.6.	Was clinical significance as well as statistical significance reported?	Yes
	8.7.	If negative findings, was a power calculation reported to address type 2 error?	No
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**		Yes
	9.1.	Is there a discussion of findings?	Yes
	9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**		Yes
	10.1.	Were sources of funding and investigators' affiliations described?	Yes
	10.2.	Was the study free from apparent conflict of interest?	Yes

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